Infection and Drug Resistance

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ORIGINAL RESEARCH

The Role of Interferon-Induced Proteins with Tetratricopeptide Repeats 1 and 2 in Sepsis-Induced Acute Liver Injury

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Background: Sepsis refers to a life-threatening organ dysfunction which can be resulted from the infection-induced dysregulated host response. A large number of inflammatory cytokines are released to act on the liver, making the liver one of the common target organs for the development of multiple organ dysfunction syndrome (MODS) in patients with sepsis. Sepsis-induced acute liver injury (SALI) can aggravate systemic disease. As a result, it is of great clinical significance to comprehend the molecular biological mechanism of SALI and to identify the markers for evaluating SALI. Interferon-induced proteins with tetratricopeptide repeats 1 and 2 (IFIT1, IFIT2) have been recognized as the anti-inflammatory factors that are widely expressed in various organs. The present study was aimed at clarifying the roles of IFIT1 and IFIT2 in the development of SALI.

Methods: A two-sample Mendelian randomization (MR) analysis was employed. Summary statistics datas were obtained from GWAS for inflammatory factors [tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6)], IFIT2, and sepsis as well as liver injury. Independent SNPs were selected as instrumental variables (IVs). Inverse variance weighted (IVW) in the MR analysis was adopted as the primary method for estimating the causal associations of inflammatory factors and IFIT2 with two diseases, and the associations of inflammatory factors with IFIT2. Additionally, weighted median method, MR-Egger and sensitivity analyses were applied in assessing the robustness of the results and ensure the result reliability. Subsequently, 119 healthy volunteers, 116 patients with sepsis and 116 SALI patients were recruited. The ELISA method was employed to quantify the expression levels of TNF-α, IL-1β, and IL-6. Additionally, qRT-PCR was conducted to measure the expression of IFIT1 and IFIT2. Furthermore, the correlations of IFIT1 and IFIT2 with inflammatory factors, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were explored.

Results: As shown by the MR analysis, the genetically predisposed sepsis was significantly associated with the risk of IL-1β, with an odds ratio (OR) of 1.069 (95% confidence interval (CI), 1.015–1.127, p = 0.0119), and negatively associated with the risk of IL-6, with an OR of 0.880 (95% CI: 0.792–0.979, p= 0.0184). Meanwhile, there were positive causal effects of IL-6 (OR = 1.269, 95% CI: 1.032–1.561, p= 0.0238), IL-1β (OR = 1.106, 95% CI: 1.010–1.211, p = 0.0299) and IFIT2 (OR = 1.191, 95% CI: 1.045–1.359, p = 0.0090) on liver injury. Additionally, there was a positive causal effect of IFIT2 (OR = 1.164, 95% CI: 1.035–1.309, p= 0.0110) on IL-1β. Upon sensitivity analyses, there was weak evidence of such effects, indicating that the findings of this study were robust and reliable. Our results revealed the elevated levels of TNF-α, IL-1β, and IL-6 in the blood samples of sepsis and SALI patients ($p < 0.0001$). Conversely, IFIT1 and IFIT2 demonstrated the significantly decreased levels in peripheral blood mononuclear cells (PBMCs) of SALI patients ($p < 0.0001$). Furthermore, the expression levels of IFIT1 and IFIT2 were both negatively correlated with ALT activity ($r = -0.3426$, $p = 0.0002$; $r = -0.3069$, $p = 0.0008$) and AST activity ($r = -0.2483$, $p = 0.0072$; $r = -0.3261$, $p = 0.0004$), respectively. Moreover, the expression of IFIT1 and IFIT2 was both negatively related to the levels of TNF-α ($r = -0.5027$, $p < 0.0001$;

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r = −0.4218, p < 0.0001), IL-1β (r = −0.3349, p = 0.0002; r = −0.4070, p < 0.0001) and IL-6 (r = −0.2734, p = 0.0030; r = −0.3536, p < 0.0001), respectively.

Conclusion: IFIT1 and IFIT2 can serve as the diagnostic markers for sepsis-related liver injury, and IFIT1 and IFIT2 may participate in the pathological process of sepsis-related liver injury by regulating inflammation and liver function.

Keywords: acute liver injury, sepsis, IFIT1, IFIT2, inflammation, Mendelian randomization

Introduction

Sepsis is recognized as the systemic inflammatory response syndrome and a significant contributor to organ failure.[1] This condition is featured by an uncontrolled systemic inflammatory response of the organism to infection (or pathogenic products), resulting in organ dysfunction, shock, and even multi-organ dysfunction[2,3]. Sepsis has been reported as the main reason for death in intensive care units (ICUs), which shows high morbidity and mortality rates.[4]

Acute liver injury (ALI) can occur in any stage of sepsis, and liver dysfunction is one of the hallmarks of the progressive development of sepsis into multi-organ dysfunction.[5] As the largest gland in the body, the liver is an organ vulnerable to sepsis.[3] It is implicated in metabolism and detoxification, and plays a more critical role than metabolic and immune homeostasis in the host defense against pathogens.[6,7] Changes in gene and protein expression and functional abnormalities are common events during the sepsis-mediated progression of ALI.[8,9] In spite of the rapid research advances, the pathophysiology of sepsis-induced ALI and the interventions to cure ALI are still limited. Hence, it is imperative to comprehensively investigate the underlying mechanisms of sepsis-induced acute liver injury (SALI) and explore the potential therapeutic strategies.

SALI involves complex pathophysiological changes, including abnormal apoptosis, inflammation, and oxidative stress.[10] Early sepsis leads to ALI due to the disruption of hemodynamics, which thus directly damages hepatocytes and hepatic tubule.[11] The excessive hepatocyte apoptosis seen in liver injury can decrease liver function or even lead to liver failure, generating microvascular destruction and immunosuppression.[12–14] However, the molecular mechanism of septic liver injury remains largely unexplored, which greatly limits the diagnostic markers of ALI. ALI is often accompanied by the release of inflammatory factors,[15,16] including tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). The interferon-induced tetrapeptide repeat (IFIT) family proteins exert a vital role in the antiviral immune response. As indicated by growing evidence, the IFIT family members are involved in an extensive range of in vivo pathophysiological processes. They actively contribute to the regulation of homeostasis and differentiation of various cells, including immune cells. Furthermore, given their significant associations with a spectrum of autoimmune diseases, they are the potential therapeutic targets, offering a promising avenue for the future treatment interventions.[17] According to previous bioinformatics analysis, multiple proteins show significantly aberrant expression in SALI, including IFIT1 and IFIT2.[18] Therefore, the roles of IFIT1 and IFIT2 in the pathogenesis and development of SALI remains uncertain, emphasizing the need for further investigation.

Hence, a two-sample Mendelian randomization (MR) analysis was carried out in this study to assess the potential causal relationships between sepsis/liver injury and inflammatory factors (TNF-α, IL-1β, and IL-6) as well as IFIT2. Subsequently, the expression levels of TNF-α, IL-1β, and IL-6 were compared among patients with sepsis, SALI and healthy controls. Additionally, the expression of IFIT1 and IFIT2 in peripheral blood mononuclear cells (PBMCs) from both SALI patients and healthy controls was also detected. Moreover, the correlations of IFIT1 and IFIT2 with inflammatory factors, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed. Moreover, the obtained findings may offer novel insights into the pathological mechanisms of SALI, and potentially identify targets for the clinical treatment of this condition (Figure 1).

Materials and Methods

MR analysis provides a distinctive approach to infer causation by utilizing genetic variations strongly associated with the exposure factor as instrumental variables (IVs) to ascertain the causal effects of the exposure on a particular outcome. The Mendelian inheritance law, which dictates the random allocation of alleles from parents to offspring during gamete

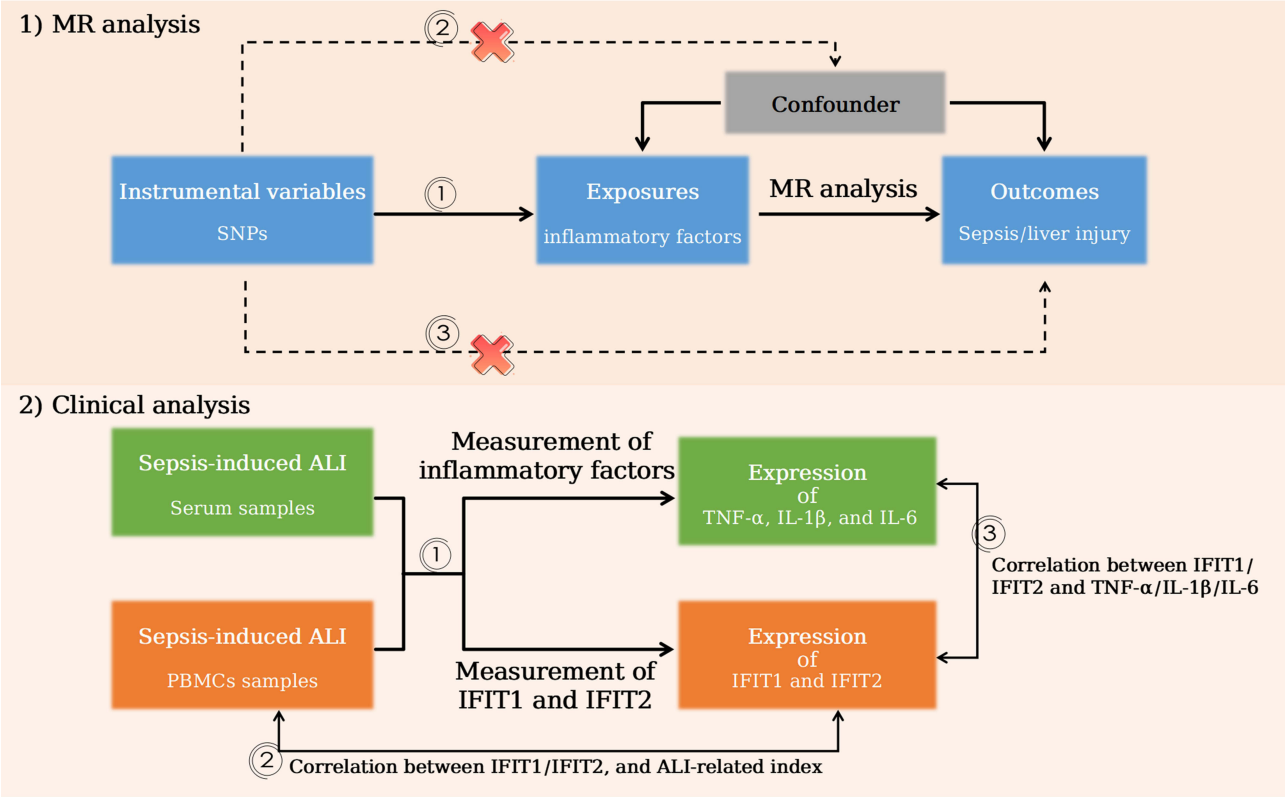


Figure 1 The schematic representation of this study.

formation, ensures that genetic variations remain unaffected by typical confounding factors such as environmental influences or individual behaviors.[19]

The temporal relationship between genetic variations and outcomes is a key feature. Consequently, MR has the potential to mitigate confounding and reverse causation biases frequently encountered in observational studies, providing more robust evidence compared to observational research.[20]

Data Collection

This MR analysis made use of summarized data extracted from published studies and databases. Ethical approval for the original studies was obtained, ensuring compliance with ethical standards. Hence, ethical approval and the need for informed consent from relevant patients were waived. The genetic data of sepsis, liver injury, IFIT2 and inflammatory factors were sourced through a meta-analysis of genome-wide association studies (GWAS) and are accessible at https://gwas.mrcieu.ac.uk/.[21] The relevant data are summarized in Table 1.

Moreover, we retrospectively collected 119 healthy volunteers who underwent a routine physical examination, 116 sepsis patients and 116 SALI patients admitted at the Emergency Department, the Affiliated Taizhou People's Hospital of

Table 1 Details of Data Sources Included in the Study

Exposures/Outcomes	GWASID	Sample Size	Number of SNPs	Sex
Sepsis	ieu-b-69	462,918	12,321,875	Males and Females
Liver injury	finn-b-K11_LIVER	218,792	16,380,466	Males and Females
TNF-α	prot-c-3722_49_2	997	501,428	Males and Females
IL-1β	prot-a-1495	3301	10,534,735	Males and Females
IL-6	ebi-a-GCST004446	8189	9,790,590	Males and Females
IFIT2	prot-a-1416	3301	10,534,735	Males and Females

Nanjing Medical University, between Jan 2019 and June 2020. The following were the inclusion criteria: (1) Sepsis patients: In accordance with the diagnostic criteria for sepsis; Hospitalization time ≥ 2 d; clear source of infection or germiculture positive evidence; (2) ALI patients: Bilirubin >34.2 μmol/L (2 mg/dL) and coagulopathy with international normalized ratio (INR)>1.5. SALI patients meet both (1) and (2) criteria. The Ethics Committee approved this project of the Affiliated Taizhou People's Hospital of Nanjing Medical University following the Declaration of Helsinki (approval number, KYKY 2019077).

Serum samples were collected from subjects by performing venous blood collection and preserved in heparin sodium-contained anticoagulation tubes until analysis. The PBMC was extracted from the peripheral blood samples.

Genetic IVs Selection

Initially, to secure a sufficient number of SNPs, we utilized a genome-wide significance threshold of $p < 1\times10^{-5}$. Subsequently, LD clumping was executed through European population reference panels, employing criteria of $R^2 < 0.001$ and a physical genetic distance > 10,000 kb to identify independent SNPs. Following this, the PhenoScanner GWAS database was employed to discern and exclude SNPs associated with potential confounding factors such as alcohol consumption and smoking. Lastly, the MR Steiger test was applied to assess the causal direction of each SNP with exposure and outcome, leading to the exclusion of SNPs exhibiting reverse causation.

MR and Sensitivity Analysis

This MR analysis adhered to the STROBE-MR guidelines. A two-sample MR analysis was conducted to assess a potential causal relationship between exposures (TNF-α, IL-1β, IL-6, and IFIT2) and the outcome (sepsis/liver injury). Another bidirectional MR analysis was conducted to assess a potential causal relationship between IFIT2 and inflammatory factors (TNF-α, IL-1β, and IL-6). The primary estimation relied on the inverse variance weighted (IVW) method, with supplementary analyses employing the MR-Egger and weight median methods.

Furthermore, sensitivity analyses were performed to evaluate potential violations of model assumptions in the MR analysis. The intercept test of MR-Egger regression was employed to identify horizontal pleiotropy. The MR-PRESSO method was utilized to detect outliers of SNPs with pleiotropic effects and provide estimates after outlier removal. Heterogeneity among SNP estimates was quantified by Cochran's Q value. Additionally, a leave-one-out analysis was carried out to assess whether a single SNP disproportionately influenced a significant effect. Funnel plots were used to evaluate the likely directionality of pleiotropy.

Measurement of Biochemical Markers

The expression levels of inflammatory factors were quantified with IL-1β ELISA kits (catalog number: H002-1-2), IL-6 ELISA kits (catalog number: H007-1-2), and TNF-α ELISA kits (catalog number: H052-1-2), which were purchased from Jiancheng Bioengineering Institute, Nanjing, China. The standard and the serum obtained by centrifugation were added to a 96-well plate and incubated for 2.5 hours at 37 °C, followed by the addition of the corresponding antibody for 1 hour, and then the termination solution was added after the addition of the substrate TMB for 30 min. The OD value was finally read at 450nm, and the corresponding expression was calculated according to the standard preparation of the calibration curve. The AU5800 automated biochemical analyzer (Beckman Coulter, USA) and supporting reagents detected AST and ALT.

qRT-PCR Analysis

The expression levels of IFIT1 and IFIT2 were quantified via qRT-PCR. Total RNA was extracted from samples by TRIzol reagent and quantified by NanoDrop. Then, cDNA was synthesized from RNA using cDNA Synthesis Kits (Beyotime, Shanghai, China, catalog number: D7182L). qRT-PCR was performed on an ABI 7500 machine using SYBR Green Mix (Thermo Fisher Scientific, Shanghai, China, catalog number: A46110) as per the instructions. PCR thermal cycling conditions were as follows: 10 min at 95°C, followed by 30 cycles of 94°C for 10s and 65°C for 20s. Relative expressions were normalized to GAPDH and quantified with the $2^{-\Delta\Delta CT}$ method. The IFIT1 and IFIT2 primer sequences

are as follows: IFIT1-F, 5' TCTAGCCAACATGTCCTCAC 3'; IFIT1-R, 5' CCTTGTAGCAAAGCCCTATCTG 3'. IFIT2-F, 5' AGCTACCGTCTGGACAACTG 3'; IFIT2-R, 5' GCTTTCTCCAAGGCTTCTTCAAC 3'.

Statistical Analysis

MR and sensitivity analyses were performed in R (version 4.2.3) using the package "TwoSampleMR" (version 0.5.7). SPSS 23.0 software (SPSS, Chicago, IL) was utilized to analyze the clinical data. The study results were presented as means ± standard deviation (SD). Shapiro–Wilk test was used to determine whether the sample data fit the normal distribution. Student's *t*-test or ANOVA were used for normal distribution, and Mann–Whitney test was used for non-normal distribution. P-value of less than 0.05 indicated statistical significance.

Results

Causal Effect of TNF-α, IL-1β, and IL-6 on Sepsis and Liver Injury

Based on the established selection criteria, certain SNPs were excluded from the analysis due to the presence of palindromic sequences, potential issues related to reverse causality, or associations with potential confounders. After applying these criteria, when the outcome was sepsis, a total of 4, 18, 10 and 16 independent SNPs were identified that reached significance levels for TNF-α, IL-1β, IL-6, and IFIT2, respectively. In addition, when liver injury was the outcome, there were 4, 14, 7 and 9 SNPs, respectively. Consequently, the causal estimation was carried out using the above SNP subset. This refined selection aimed to ensure the inclusion of SNPs that met the specified criteria and were most relevant to the investigation, enhancing the precision and reliability of the causal analysis for each inflammatory factor.

The preliminary Results from the IVW method indicated a negative causal effect of IL-6 (OR = 0.880, 95% CI: 0.792–0.979, p= 0.0184) on sepsis. Conversely, there was a positive causal effect of IL-6 (OR = 1.269, 95% CI: 1.032–1.561, p= 0.0238) on liver injury. There were positive causal effects of IL-1β on sepsis (OR = 1.069, 95% CI: 1.015–1.127, p = 0.0119) and liver injury (OR = 1.106, 95% CI: 1.010–1.211, p = 0.0299). Meanwhile, TNF-α shown no causal relationship on sepsis (OR = 1.020, 95% CI: 0.952–1.093, p = 0.5701) and liver injury (OR = 1.025, 95% CI: 0.887–1.183, p = 0.7386). Interestingly, there was no causal effect of IFIT2 on sepsis (OR = 0.993, 95% CI: 0.936–1.053, p = 0.8152), while it shown a positive causal relationship on liver injury (OR = 1.191, 95% CI: 1.045–1.359, p = 0.0091). These findings were consistent across other methods such as MR-Egger and the Weighted Median method (Figure 2 and Tables S1–8). The scatter plots visually represented the specific effects of each method on the outcome database, providing additional support for the observed causal relationships (Figures S1–8).

Causal Effect Between TNF-α, IL-1β, and IL-6 on IFIT2

In the bidirectional MR analysis, after applying the above criteria, when the outcome was IFIT2, a total of 4, 13 and 5 independent SNPs were identified that reached significance levels for TNF-α, IL-1β, and IL-6, respectively. In addition, when IFIT2 was the exposure, there were 11, 12 and 5 SNPs, respectively. There were no causal effects of TNF-α (OR = 0.927, 95% CI: 0.823–1.045, p = 0.2154), IL-1β (OR = 0.999, 95% CI: 0.890–1.120, p = 0.9803), and IL-6 (OR = 1.088, 95% CI: 0.875–1.352, p = 0.4479) on IFIT2. Meanwhile, IFIT2 shown no causal relationship on TNF-α (OR = 0.979, 95% CI: 0.744–1.288, p = 0.8784) and IL-6 (OR = 0.937, 95% CI: 0.840–1.044, p = 0.2399). Conversely, there was a positive causal effect of IFIT2 (OR = 1.164, 95% CI: 1.035–1.309, p= 0.0110) on IL-1β (Figures 3, S9–14 and Tables S9–14).

To further confirm the causal association, we conducted extensive sensitivity analyses. The Cochran Q test did not reveal statistically significant heterogeneity among the estimates of individual SNPs, indicating consistent results across different SNPs. Furthermore, the MR-Egger regression analysis did not detect statistically significant pleiotropy, suggesting that no significant genetic factors influenced both sepsis and the inflammatory factors. (Tables 2 and S15–56).

Additionally, leave-one-out analyses were performed to validate the observed causal effect and explore the potential impact of individual instrumental variables (IVs) on the results. The leave-one-out analysis revealed that the causal effect of sepsis and the inflammatory factors was not driven by any single SNP (Figures S1–14).

Outcomes	Exposures	Methods	SNPs(N)	OR (95% CI)	P value
Sepsis	TNF-α	MR Egger	4	0.998(0.688-1.448)	0.9924
		Weighted median	4	1.021(0.943-1.105)	0.6055
		IVW	4	1.020(0.952-1.093)	0.5701
	IL-1β	MR Egger	18	1.053(0.926-1.198)	0.4442
		Weighted median	18	1.069(0.991-1.155)	0.086
		IVW	18	1.069(1.015-1.127)	**0.0119**
	IL-6	MR Egger	10	0.875(0.703-1.088)	0.2634
		Weighted median	10	0.871(0.755-1.006)	0.0605
		IVW	10	0.880(0.792-0.979)	**0.0184**
	IFIT2	MR Egger	16	1.018(0.896-1.162)	0.7989
		Weighted median	16	1.010(0.936-1.088)	0.8151
		IVW	16	0.993(0.936-1.053)	0.8152
Liver injury	TNF-α	MR Egger	4	0.809(0.271-2.415)	0.7404
		Weighted median	4	0.997(0.874-1.137)	0.9626
		IVW	4	1.025(0.887-1.183)	0.7386
	IL-1β	MR Egger	14	1.173(0.946-1.455)	0.1707
		Weighted median	14	1.155(1.025-1.301)	0.0177
		IVW	14	1.106(1.010-1.211)	**0.0299**
	IL-6	MR Egger	7	1.388(0.884-2.180)	0.2141
		Weighted median	7	1.197(0.918-1.560)	0.1837
		IVW	7	1.269(1.032-1.561)	**0.0238**
	IFIT2	MR Egger	9	1.069(0.776-1.473)	0.6946
		Weighted median	9	1.131(0.941-1.359)	0.1891
		IVW	9	1.191(1.045-1.359)	**0.0091**

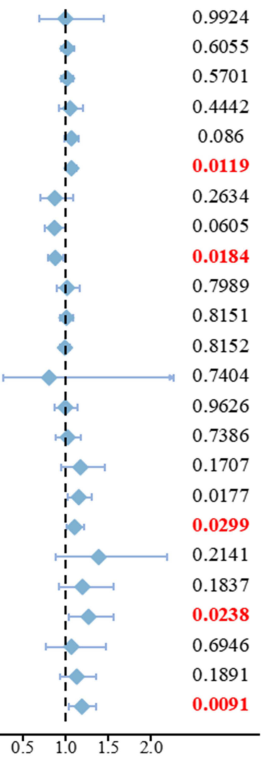


Figure 2 The causal effect of TNF-α, IL-1β, and IL-6 on sepsis and liver injury.

Outcomes	Exposures	Methods	SNPs(N)	OR (95% CI)	P value
IFIT2	TNF-α	MR Egger	4	0.938(0.490-1.795)	0.8639
		Weighted median	4	0.917(0.796-1.057)	0.2329
		IVW	4	0.927(0.823-1.045)	0.2154
IFIT2	IL-1β	MR Egger	13	1.321(1.018-1.714)	0.0604
		Weighted median	13	1.050(0.904-1.219)	0.5251
		IVW	13	0.999(0.890-1.120)	0.9803
IFIT2	IL-6	MR Egger	5	1.060(0.680-1.652)	0.8137
		Weighted median	5	1.121(0.848-1.482)	0.4232
		IVW	5	1.088(0.875-1.352)	0.4479
TNF-α	IFIT2	MR Egger	11	0.750(0.298-1.883)	0.555
		Weighted median	11	0.944(0.665-1.341)	0.7495
		IVW	11	0.979(0.744-1.288)	0.8784
IL-1β	IFIT2	MR Egger	12	1.115(0.853-1.459)	0.4424
		Weighted median	12	1.270(1.087-1.484)	0.0025
		IVW	12	1.164(1.035-1.309)	**0.0111**
IL-6	IFIT2	MR Egger	5	0.871(0.654-1.160)	0.4152
		Weighted median	5	0.972(0.849-1.113)	0.6833
		IVW	5	0.937(0.840-1.044)	0.2399

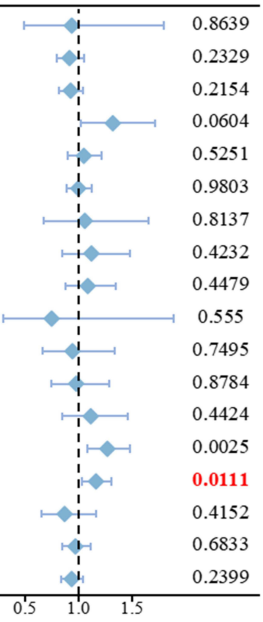


Figure 3 The causal effect between TNF-α, IL-1β, and IL-6 and IFIT2.

Table 2 Heterogeneity and Pleiotropy Analyses

Outcomes	Exposures	Heterogeneity			Pleiotropy Test		
		Q	Q df	Q *P* value	Egger Intercept	SE	*P* value
Sepsis	TNF-α	0.54	3	0.910	0.010	0.081	0.917
	IL-1β	14.72	17	0.615	0.004	0.014	0.799
	IL-6	8.89	9	0.448	0.001	0.014	0.948
	IFIT2	4.10	14	0.995	−0.005	0.012	0.680
Liver injury	TNF-α	6.63	3	0.085	0.099	0.231	0.710
	IL-1β	6.12	13	0.942	−0.012	0.020	0.562
	IL-6	1.74	6	0.942	−0.010	0.022	0.681
	IFIT2	3.19	7	0.867	0.028	0.038	0.491
IFIT2	TNF-α	11.22	9	0.261	0.038	0.063	0.566
	IL-1β	8.67	10	0.564	0.009	0.027	0.735
	IL-6	1.66	3	0.647	0.014	0.026	0.628
TNF-α	IFIT2	0.526	2	0.769	−0.005	0.143	0.976
IL-1β	IFIT2	10.04	11	0.527	−0.059	0.026	0.043
IL-6	IFIT2	3.32	3	0.345	0.005	0.035	0.902

Increased Expression of Inflammatory Factors in Sepsis and SALI Serum Samples

Furthermore, the expression of inflammatory factors between SALI patients, sepsis patients and healthy controls were compared. As shown in Figure 4, TNF-α, IL-1β, and IL-6 were all increased in serum of sepsis and SALI compared with healthy control ($p<0.01$).

IFIT1 and IFIT2 Expressions Were Decreased in SALI PBMCs Samples

To investigate the roles of IFIT1 and IFIT2 in the pathogenesis and development of sepsis-induced ALI, the levels of IFIT1 and IFIT2 in PBMCs were measured by qRT-PCR. The results in Figure 5A and B illustrated that IFIT1 and IFIT2 were significantly decreased in PBMCs from SALI patients compared with healthy controls. Subsequently, the ROC analysis confirmed that the area under the curves (AUCs) of IFIT1 and IFIT2 in discriminating SALI from healthy controls were 0.8649 (95% CI = 0.88176 to 0.9123) and 0.9368 (95% CI = 0.8999 to 0.9737), respectively (Figure 5C and D).

Correlation Between IFIT1, IFIT2, and ALI-Related Index

The baseline indicators of SALI patients and healthy controls were recorded. As shown in Figure 6A–D, the expression level of IFIT1 was negatively correlated with alanine aminotransferase (ALT) activity (r=−0.3426, $p=0.0002$) and aspartate aminotransferase (AST) activity (r=−0.2483, $p=0.0072$); on the other hand, IFIT2 was also negatively correlated with ALT activity (r=−0.3069, $p=0.0008$) and AST (r=−0.3261, $p=0.0004$) activity.

Correlation Between IFIT1, IFIT2 and Levels of Inflammatory Factors in SALI Serum Samples

Furthermore, the correlation between IFIT1, IFIT2 and levels of inflammatory factors in SALI serum samples were analyzed. Results showed that the expression of IFIT1 were negatively correlated with the levels of TNF-α (r=−0.5027, $p<0.0001$), IL-1β (r=−0.3349, $p=0.0002$) and IL-6 (r=−0.2734, $p=0.0030$), respectively; moreover, the expression level of IFIT2 was also negatively correlated with TNF-α (r=−0.4218, $p<0.0001$), IL-1β (r=−0.4070, $p<0.0001$) and IL-6 (r=−0.3536, $p<0.0001$), respectively (Figure 7).

Discussion

In the acute stage of sepsis, inflammatory factors, mainly secreted by immune cells, play an essential role in the host defense against pathogens. However, the inflammatory factors are dynamic, which only indicate the levels of inflammatory factors in a certain stage. While the levels of circulating inflammatory factors in liver injury are also unknown.

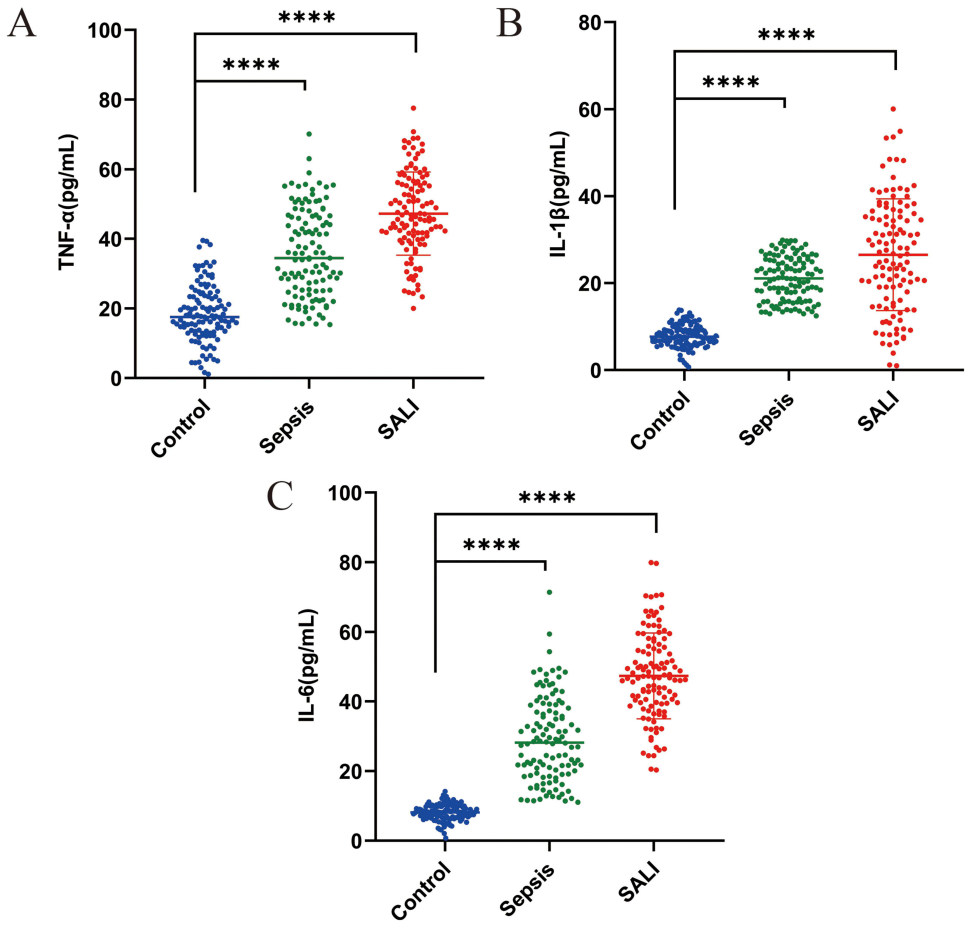


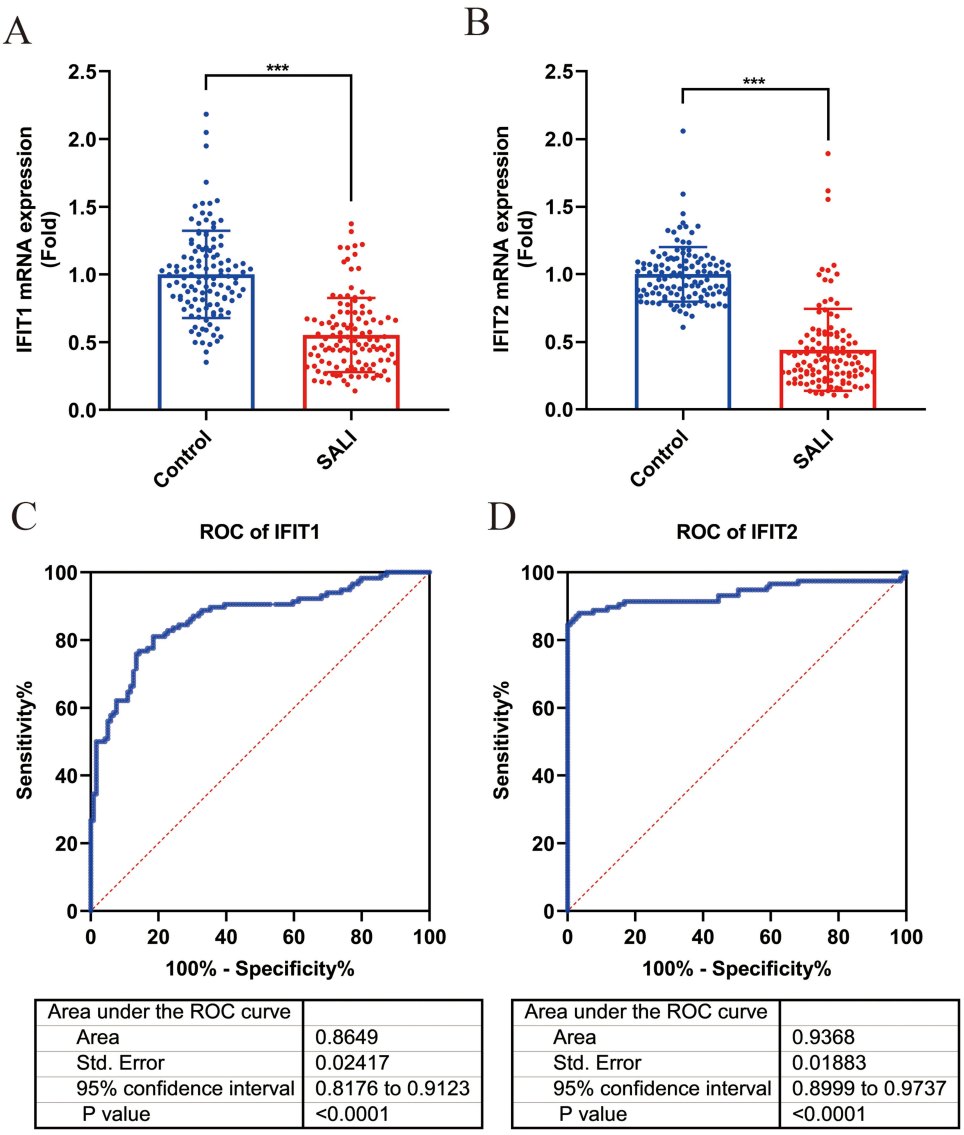
Figure 4 Elevated expression of TNF-α, IL-1β, and IL-6 in serum of patients with sepsis and SALI. (**A**) Comparison between TNF-α expression in serum of patients with sepsis, SALI and healthy controls(*** *p<0.00001). (**B**) Comparison between IL-1β expression in serum of patients with sepsis, SALI and healthy controls (****p<0.00001). (**C**) Comparison between IL-6 expression in serum of patients with sepsis, SALI and healthy controls (****p<0.00001).

Hence, in this study, the two-sample MR method was adopted for exploring the potential causal associations between the circulating levels of three common factors and the risk of sepsis and liver injury. Our analysis uncovered suggestive evidence, indicating that the circulating levels of genetically predisposed IL-6 and IL-1βwere associated with sepsis and liver injury. These findings deepen our knowledge of the intricate interplay between genetic susceptibility to inflammatory factors and the development of sepsis and liver injury.

An intriguing finding emerged during MR analysis, suggesting that IL-6 exhibited a negative correlation with sepsis, which was indicative of a protective role, contrary to the expected elevation in inflammatory factors. IL-6, a widely recognized cytokine and commonly measured acute-phase reactant, introduces complexity to this narrative. Furthermore, the reduction of IL-6 signaling is associated with a moderately increased risk of infection, as demonstrated in randomized trials on IL-6 antagonists.[22] However, this association is nuanced and changeable by the apparent benefits observed in some severe infections with a reduced IL-6 activity.[23]

Furthermore, previous bioinformatics analysis revealed that multiple proteins showed significantly aberrant expression in SALI, including IFIT1 and IFIT2.[18] Due to the limitations of the GWAS database, we only performed relevant MR studies for IFIT2. This analysis uncovered suggestive evidence, indicating that the genetically predisposed IFIT2 was associated with the risk of liver injury. Additionally, IFIT2 showed a positive causal effect on IL-1β.

The obtained findings offer valuable insights into the conceivable impact of specific cytokines on the susceptibility to sepsis and liver injury, as discovered through a meticulous genetic approach. Nonetheless, it is imperative to underscore that further clinical research is warranted to validate and expand our results. Therefore, we delved deeper into the expression of



Area under the ROC curve	
Area	0.8649
Std. Error	0.02417
95% confidence interval	0.8176 to 0.9123
P value	<0.0001

Area under the ROC curve	
Area	0.9368
Std. Error	0.01883
95% confidence interval	0.8999 to 0.9737
P value	<0.0001

Figure 5 IFIT1 and IFIT2 were prominently downregulated in PBMCs from patients with SALI. (**A**) Expression of IFIT1 in PBMCs from SALI patients and healthy volunteers (***p<0.0001). (**B**) Expression of IFIT2 in PBMCs from SALI patients and healthy volunteers (***p<0.0001). (**C**) ROC analysis of IFIT1 in discriminating SALI from healthy controls. (**D**) ROC analysis of IFIT2 in discriminating SALI from healthy controls.

TNF-α, IL-1β, and IL-6. Our clinical results were consistent with the majority of studies, revealing the elevated levels of TNF-α, IL-1β, and IL-6 in patients with sepsis and SALI. The emerging consensus points to the overactivation of the pro-inflammatory system in SALI, in which pro-inflammatory cytokines, which include TNF-α, IL-1β, and IL-6, exert a critical role in the pathogenesis of ALI.[24] It becomes evident that inhibiting pro-inflammatory cytokines holds promise for alleviating the severity of ALI.[25] Inhibition of TNF-α is demonstrated to alleviate pyroptosis in LPS-triggered ALI.[26] Furthermore, the inhibitory effects of B. rapa polysaccharides on the secretion of IL-1β and IL-6 have been highlighted, which offers a potential avenue for ameliorating the development of ALI.[27] These findings underscore the multifaceted nature of cytokine involvement in SALI, opening avenues for therapeutic exploration and further research.

IFIT1 and IFIT2, the proteins characterized by tetrapeptide repeat sequences, are initially identified to be induced by interferon treatment, which exhibit inhibitory effects on viral replication and translation initiation.[28,29] Notably, Chang et al[30] proposed that the enforced expression of IFIT1 conferred protection against the LPS-induced fatal hepatitis, thereby contributing to the mitigation of inflammatory liver diseases. Additionally, some studies have revealed that the

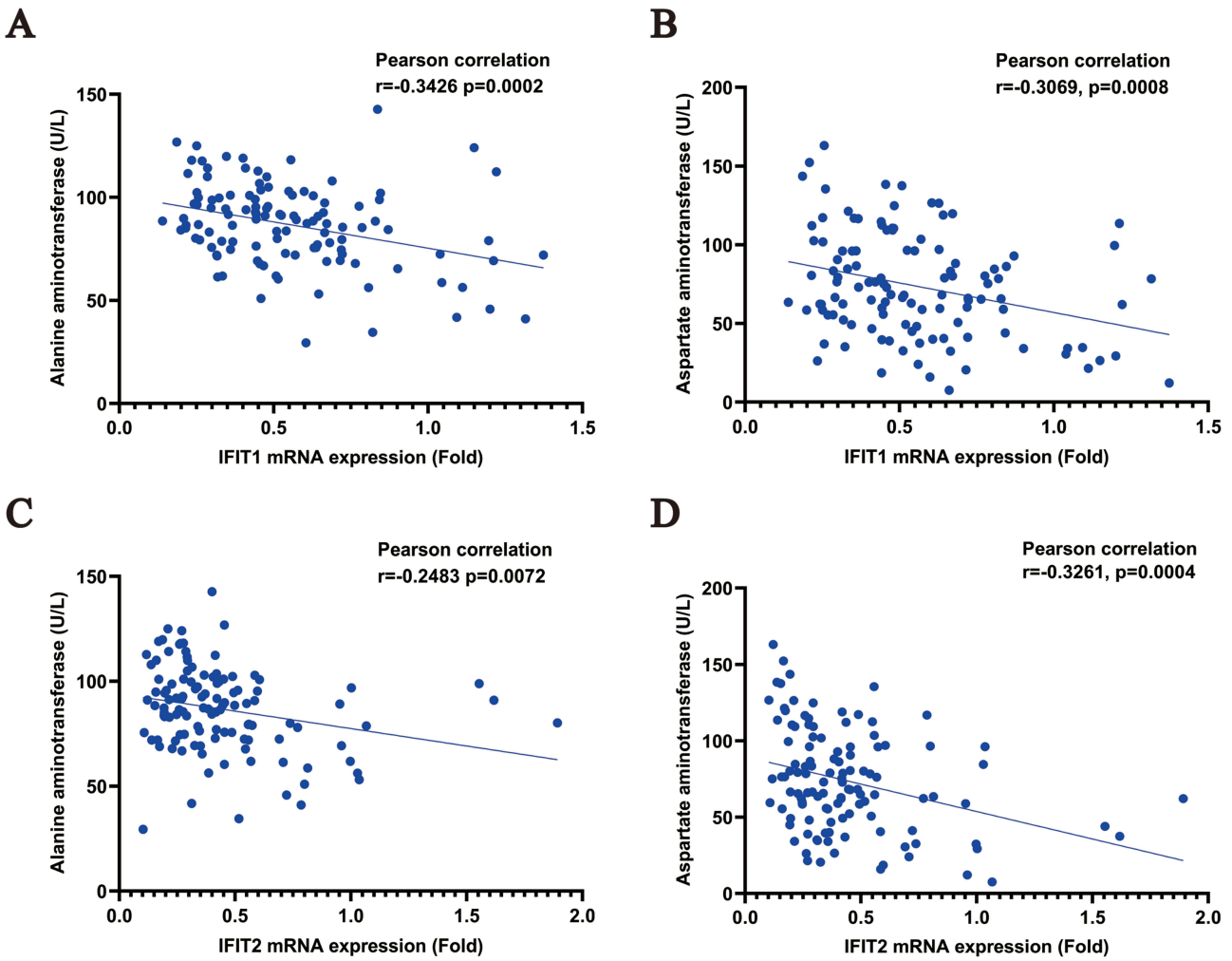


Figure 6 Correlation between IFIT1, IFIT2 expressions, and ALI-related scores. (**A**) Correlation analysis between IFIT1 expression and ALT activity. (**B**) Correlation analysis between IFIT1 expression and AST activity. (**C**) Correlation analysis between IFIT2 expression and ALT activity. (**D**) Correlation analysis between IFIT2 expression and AST activity.

elevated levels of IFIT1 and IFIT2 enhance antiviral activity, thereby playing a role in both HCV infection and treatment.[17]

In alignment with previous research, our study sheds lights on the notable reduction in the expression of IFIT1 and IFIT2 in PBMCs derived from SALI patients. This underscores the potential of IFIT1 and IFIT2 as the high-sensitivity diagnostic biomarkers for SALI. Concurrently, our investigation established a negative correlation between the expression levels of IFIT1 and IFIT2 and the activities of AST and ALT. This suggested a close association of IFIT1 and IFIT2 with the development of SALI. These comprehensive results significantly contribute to an enhanced understanding of the potential diagnostic and prognostic roles of IFIT1 and IFIT2 in SALI.

Moreover, our results suggested that the serum levels of TNF-α, IL-1β, and IL-6 notably elevated in ALI patients. Notably, the expression levels of IFIT1 and IFIT2 exhibited a negative relationship to the serum expression of these cytokines. This was in contrast to the positive correlation between IFIT2 and IL-1β suggested by the MR analysis described above. The unexpected results from our study suggested a complex relationship between these conditions, highlighting the need for further research to validate and understand the underlying mechanisms behind these associations, and address the inconsistencies. Collectively, these findings suggest a potential protective role of IFIT1 and IFIT2 against the inflammatory response in SALI.

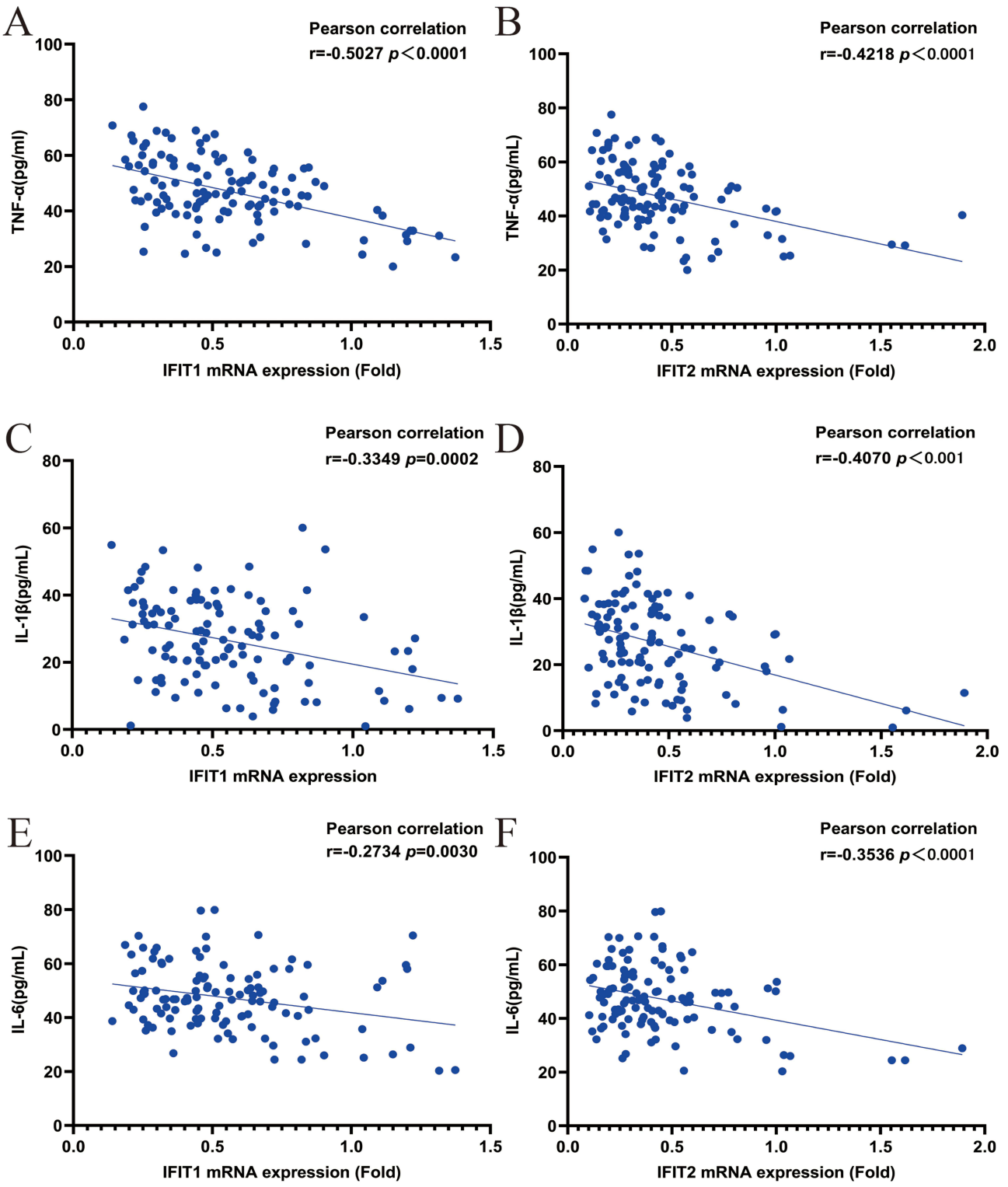


Figure 7 Correlation between IFIT1, IFIT2 expressions and serum level of inflammatory factors. (**A**) Correlation analysis between IFIT1 and TNF-α. (**B**) Correlation analysis between IFIT2 and TNF-α. (**C**) Correlation analysis between IFIT1 and IL-1β. (**D**) Correlation analysis between IFIT2 and IL-1β. (**E**) Correlation analysis between IFIT1 and IL-6. (**F**) Correlation analysis between IFIT2 and IL-6.

Conclusion

In summary, our study reveals the down-regulated levels of IFIT1 and IFIT2 in SALI patients, highlighting their potential as the viable diagnostic biomarkers for SALI. Moreover, the observed down-regulation of IFIT1 and IFIT2 suggests a protective role against liver injury by attenuating the inflammatory response in SALI.

Data Sharing Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The Ethics Committee approved this project of Taizhou People's Hospital affiliated to Nanjing Medical University following the Declaration of Helsinki (approval number, KYKY 2019077).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. Zhipeng Liu and Xinyu Yuan contributed equally to this work and shared the first authorship. Jun Wang and Shanshan Xue contributed equally to conceived and designed the experiments and shared the corresponding authors.

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Disclosure

The authors report no conflicts of interest in this work.

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